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JRC F.5/CvH/MGH/AS/Ares

Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003

Mugwort tincture

(FAD-2010-0401; CRL/100351)

European Union Reference Laboratory
Feed Additives

Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	**FAD-2010-0401 - CRL/100351**
Name of Feed Additive:	***Mugwort tincture***
Phytochemical marker(s):	**-**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
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Report checked by: Date:	**Zigmas Ezerskis** **18/07/2019**
Report approved by: Date:	**Christoph von Holst** **18/07/2019**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for the botanically defined *Mugwort tincture* under the category/functional group (2 b) "sensory additives"/"flavouring compounds", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, the *feed additive* is sought to be used for all animal species and categories.

The *feed additive* is a mixture of naturally occurring chemical components including total polyphenols, total phenolic acids, chlorogenic acid, alpha- and beta- thujones and eucalyptol as the major constituents, and it is intended to be incorporated directly into *feedingstuffs* or through flavouring premixtures without minimum or maximum limits.

The Applicant did not provide a method for the determination of the phytochemical marker, but submitted other methods aiming at the identification/characterisation of the *feed additive* (*Mugwort tincture*).

The Applicant proposed to characterise the *feed additive* (*Mugwort tincture*) by determination of loss on drying, ash content (measured by gravimetry), total polyphenols (measured by spectrophotometry), total phenolic acids, chlorogenic acid, alpha- and beta- thujones and eucalyptol (measured by high performance thin layer chromatography (HPTLC)). According to the Applicant, the use of the HPTLC profiles as a fingerprint of the *feed additive* is more reliable than the analysis of individual phytomarkers at an established range.

For the identification/characterisation of the *feed additive* the EURL considers the methods based on gravimetry, spectrophotometry and high performance thin layer chromatography (HPTLC) proposed by the Applicant as fit-for-purpose.

Furthermore, the Applicant did not provide experimental data or analytical method for the determination of *Mugwort tincture* in *premixtures* and *feedingstuffs* as the unambiguous determination of the *feed additive* added to the matrices is not achievable experimentally.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

KEYWORDS

Mugwort tincture, sensory additives, flavouring compounds, all animal species

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (new feed additive) for the botanically defined *Mugwort tincture* under the category/functional group (2 b) "sensory additives"/"flavouring compounds", according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1-3]. Specifically, the *feed additive* is sought to be used for all animal species and categories [2-4].

The *feed additive* is a hydro alcoholic brown extract (75 % water and 25 % ethanol) of mugwort aerial parts obtained after maceration [5] containing a mixture of naturally occurring chemical components including total polyphenols, total phenolic acids, chlorogenic acid, alpha- and beta- thujones and eucalyptol as major constituents [5].

The *feed additive* is intended to be incorporated directly into *feedingstuffs* or in combination with other flavouring substances (flavouring premixtures) without minimum or maximum limits. However, the Applicant recommends its incorporation at minimum levels ranging from 0.7 to 24 mg *feed additive* /kg *feedingstuffs* and maximum levels from 5.1 to 65 mg *feed additive* /kg *feedingstuffs* depending on the target animal species [4].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761, on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and the tasks of the European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *Mugwort tincture* and their suitability to be used for official controls in the frame of the authorisation were evaluated.

3. EVALUATION

Description of the analytical methods for the determination of the active substance in the feed additive, premixtures, feedingstuffs and when appropriate water (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

The Applicant did not provide a method for the determination of the phytochemical marker(s), but submitted other methods aiming at the identification/characterisation of the *feed additive* (*Mugwort tincture*).

Furthermore, the Applicant did not provide experimental data or analytical method for the determination of *Mugwort tincture* in *premixtures* and *feedingstuffs* as the unambiguous determination of the *feed additive* added to the matrices is not achievable experimentally.

Methods of analysis for the determination of the residues of the additive in food (section 2.6.2 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

An evaluation of corresponding methods of analysis is not relevant for the present application.

Identification/Characterisation of the feed additive (section 2.6.3 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

The Applicant proposed to characterise the *feed additive* (*Mugwort tincture*) by the determination of loss on drying, ash content, total polyphenols, total phenolic acids, chlorogenic acid, alpha- and beta- thujones, and eucalyptol [6].

For the determination of loss on drying and ash content in the *feed additive* the Applicant proposed the use of gravimetric methods.

For the determination of total polyphenols the Applicant proposed a spectrophotometry method based on the European Pharmacopoeia monograph [7].

For the determination total phenolic acids [8], chlorogenic acid [9], alpha- and beta- thujones [10] and eucalyptol [11] the Applicant proposed a high performance thin layer chromatography (HPTLC) method. This method is based on the one described in the French Pharmacopoeia for the determination of chlorogenic acid in Mugwort tincture [12].

The Applicant has provided the result of the analysis of five different batches of the *feed additive* (*Mugwort tincture*) characterised by applying the methods mentioned above. These analyses led to average values of 1.7 % for the loss on drying; 0.3 % for the ash content; 0.1 % of total polyphenols; 0.03 % of total phenolic acids including 0.006 % of chlorogenic acid; less than 0.005 % of alpha- and beta- thujones and 0.0011 % of eucalyptol [5].

However, according to the Applicant the use of the HPTLC profiles of the phenolic fraction as a fingerprint of the additive is considered a more reliable way to ensure the absence of adulteration and thus preferred than the analysis of individual phytomarkers at an established range.

For the identification/characterisation of the *feed additive* the EURL considers the above mentioned methods based on gravimetry, spectrophotometry and high performance thin layer chromatography (HPTLC) as fit-for-purpose.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for the characterisation of *feed additive* (*Mugwort tincture*) the gravimetric methods for the determination of loss on drying and the ash content; the spectrophotometric method for the determination of total polyphenols; and the method based on high performance thin layer chromatography (HPTLC) for the determination of total phenolic acids, chlorogenic acid, alpha- and beta- thujones and eucalyptol.

Recommended text for the register entry (analytical method)

For the characterisation of the *feed additive* (*Mugwort tincture*):

- gravimetric method for the determination of loss on drying and the ash content
- spectrophotometric method for the determination of total polyphenols content
- high performance thin layer chromatography (HPTLC) method for the determination of total phenolic acids, chlorogenic acid, alpha- and beta- thujones and eucalyptol

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Mugwort tincture* have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

6. REFERENCES

[1] *Application, Reference SANCO/D/2: FORW. APPL. 1831/(0010) (10517)-2011

[2] *Application form, Annex I, Submission No. 1288116422339-1176

[3] *Application, Proposal for Register Entry – Annex A

[4] Supplementary information -Section II: 2.5.1 Proposed mode of use in animal nutrition

[5] Supplementary information - Section II: 2.1.3 Qualitative and quantitative composition

[6] Supplementary information – Annex II-2

[7] European Pharmacopoiea, Chapter 2.8.14 Determination of tannins in herbal drugs

[8] Supplementary information – Annex II-5

[9] Supplementary information – Annex II-6

[10] Supplementary information – Annex II-7

[11] Supplementary information – Annex II-8

[12] French Pharmacopoiea, Mugwort for homoeopathic preparations Artemisia vulgaris for homoeopathic preparations

*Refers to Dossier no: FAD-2010-0401

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the European Union Reference Laboratory for Feed Additives, JRC, Geel, Belgium. This report is in accordance with the opinion of the consortium of National Reference Laboratories as referred to in Article 6(2) of Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761.

8. ACKNOWLEDGEMENTS

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- Instytut Zootechniki - Państwowy Instytut Badawczy, Krajowe Laboratorium Pasz, Lublin (PL)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Wageningen Food Safety Research[i] (WFSR) (NL)
- LGC Ltd, Teddington (UK)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural**.** Generalitat de Catalunya, Cabrils (ES)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)

Name and address according to according COMMISSION IMPLEMENTING REGULATION (EU) 2015/1761:

[i] RIKILT Wageningen UR, Wageningen.